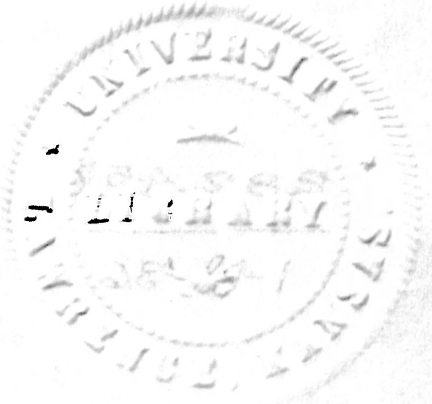
Studies on Complement

by Noble P. Sherwood

1921

Submitted to the Graduate Faculty and the Board of Administration of the University of Kansas in Partial Fulfiliment of the Requirements for the Degree of Doctor of Philosophy.



STUDIES ON COMPLEMENT

BY

NOBLE P. SHERWOOD

SUBMITTED TO THE GRADUATE FACULTY AND THE BOARD OF ADMINISTRATION OF THE UNIVERSITY OF KANSAS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

APPROVED

Frank W. Blackmar

DEAN OF THE GRADUATE SCHOOL

APPROVED BY

H. Gideon Wells

PROFESSOR OF PATHOLOGY
UNIVERSITY OF CHICAGO

A. P. W. Ahl

Professor of Pathology
School of Medicine
University of Kansas
Rosedale, Kansas.

TABLE OF CONTENTS

INTRODUCTION

The author realizes that perhaps a word of explanation might be of value showing that there was a definite object and a reasonable degree of continuity in these studies.

An interesting activating thermolabile substance discovered by Buchner and named alexin by both Buchner and Bordet and complement by Ehrlich has been the subject of active investigation for many years. Metchnikoff and his school have felt that it was produced by the leucocytes and have described two complements; one a microcytase, the bactericidal complement, and the other a macrocytase, or hemolytic complement. Ehrlich and his school however have been opposed to this view and have held that complement was produced by the liver. The different methods which have been used to investigate Ehrlich's view may be stated as follows:

1. Administration of chemical compounds that would produce necrosis of liver tissue.
2. Impairment of liver function by anastomosing the portal vein with the inferior vena cava.
3. Complete extirpation of the liver.
4. A few perfusion experiments.

The author realized that the men who investigated the question along the lines indicated must have accepted as fairly well established that complement is probably a secretion and is present in the circulating plasma. The present work does not attempt to investigate these two points. A great deal of research already published seems to uphold this contention.

He did feel that the experimental work purporting to show that the liver was the source of complement, was open to some fundamental objections as follows:

1. The complement titrations were made upon animals that survived only a few hours.
2. That no study of the effect upon body metabolism or complement directly by the compounds used had been attempted.
3. That an investigation was not made to determine the effect of abnormal amounts of normal by-products of metabolism upon complement.

A great deal of valuable information has been obtained as to its role in immunity reactions, composition, prevalence, effect of light, heat, shaking, inert substances, inorganic salts, etc. upon

its activity, but no satisfactory information has been obtained as to its origin and very little as to the effect of organic compounds upon its activity.

In the hope of throwing more light upon this rather obscure and rather important question the present studies were carried out. In them you will find an attempt to answer the following questions:

1. Is there a drop in complement content of dogs that have had produced an impairment of their liver function as a result of an Eck-fistula operation?
2. If there is a drop what is its time of duration and is it associated with changes in the liver?
3. Is the drop associated with anything else such as type of anesthetic used, duration of anesthesia, shock, metabolism changes resulting from the anesthetic, etc.?
4. Could any of the chemical substances used by some to produce necrosis of liver tissue have any effect upon complement directly?

5. Could any normal organic substances if present in abnormal amount affect complement?
6. Would the complements obtained from different animals be similarly affected?
7. Do the results warrant the assumption that the liver is the sole source of complement.
8. Is there any practical application of these results other than the ones mentioned.

In order to at least partially answer these questions the work has been handled in three studies and a final resume made of the findings. The author realizes the dangers of error in interpreting experimental data and has endeavored to be conservative in his conclusions. He therefore hopes that this may be kept in mind in judging these published results.

In closing he might say in explanation of the apparent joint authorship of the first paper that the following statement from Dr. Smith will explain the conditions as they existed:

My dear Dean Blackmar:

Relative to the participation which Mr. West andI had in the paper entitled "The Complement Content of Eck-Fistula Dogs" I am very glad to make the following statement: I wished to study the nitrogen metabolism of Eck-Fistula dogs and at Dr. Matthews' suggestion we arranged to use the same animals since Mr. Sherwood's study of the complement content of the blood would in no manner interfere with our research work. The Eck-Fistula operations were done in the operating room of the department of Physiology and Mr. Sherwood did his immunological studies in the immunity laboratory of the department of Bacteriology. As an evidence of his appreciation for our assistance along the lines of surgery he granted Mr. West and myself credit on the paper which he published.

Yours very truly,

Clarence C. Smith, M.D.

(Signed)

STUDIES I

THE COMPLEMENT CONTENT OF ECK-FISTULA DOGS

Many investigators have attempted to associate the liver with the production of complement.

Ehrlich and Morgenroth[1] observed a diminution of complement in dogs subjected to phosphorus poisoning with subsequent degeneration of the liver. Extirpation of the liver has been attempted by Nolf[2] and others, but in view of the almost insurmountable difficulties of the operation the work has been wholly unsatisfactory. Nolf next attempted to establish Eck's fistula in rabbits. Anastomosing the vena cava with the portal vein he obtained an immediate drop in complement, and he concluded from this that the liver played an important role in the production of the complement. His work was later confirmed by Muller,[3] who also perfused serum through the liver and observed an increase in complement. Liefmann[4] was unable to confirm the results obtained by Muller. As a further check on the work he extirpated the liver from frogs, but observed no diminution of complement, the animals living, in some cases, one week.

More recently Dick[5] produced necrosis

of the liver in dogs by means of chloroform poisoning, as suggested by Richards and Howland.[6] He observed a progressive drop in complement, lasting until the death of the animals some 48 hours afterwards. The complement content of normal dog serum was observed to vary from one-third to one-twelfth that of guinea-pig serum. The complement content of individual dogs showed a similar variation when the serum was titrated from time to time. In the animals suffering from chloroform poisoning, the complement content dropped to one-forty-fifth that of guinea-pig serum in 46 hours, just before the death of the animals.

Dick also produced destruction of liver cells by means of hydrazin sulfate. At the beginning of the experiment the complement content was one-fifth, after 48 hours it was one-tenth, while after 72 hours it was one-fortieth that of guinea-pig serum. The dog died a few hours later. Extensive necrosis of the liver was found microscopically. Dick concludes that complement is a proteolytic ferment which is either formed in the liver or is dependent on liver activity for its presence in the blood.

It will be observed that in all the experiments cited, the animals died within a few

days after the work was started. In the work of Nolf and others on Eck's fistula only 3 or 4 hours elapsed before the animals succumbed, while Dick's chloroformed animals lived only about 48 hours.

In view of the fact that dogs in which Eck's fistula had been established by Dr. S. A. Matthews and one of us of the department of physiology, were surviving in good health almost indefinitely, it was thought worth while to study the complement content of these animals and to compare it with the progressive and noticeable atrophy, fatty degeneration and infiltration, and necrosis of the liver which followed the operation. For this work those dogs were selected which had no normal antihuman or antisheep hemolysins in their sera.

Blood was obtained from the external saphenous vein from one to several times preceding the operation and the complement content determined and compared with that of guinea-pig serum. Two hundredths cubic centimeter, that is, 0.2 cc. of a 1:10 dilution, of guinea-pig complement was adopted as a unit of comparison.

The estimation of complement was carried out as follows:

At first both antihuman and antisheep hemolytic amboceptors from rabbits immunized against

the respective cells were employed. These were titrated to determine the unit of each. In the case of the antihuman hemolytic amboceptor, we used the method of titration recommended by Noguchi[7] and others. Each hemolytic test tube contained 0.1 c.c. of a 10% suspension of washed human red blood corpuscles, 0.1 c.c. of its respective dilution of amboceptor, 0.5 c.c. of a 1:10 dilution of active guinea-pig complement, and enough physiologic salt solution to make the final volume 1 c.c. These tubes were incubated in a water bath at 37.5 C. for 30 minutes. It was found that the tube containing 0.1 cc. of 1:120 dilution of amboceptor held the highest dilution showing complete hemolysis. This wasconsidered the titer of the serum. In titrating dog complement, each hemolytic tube contained 0.1 c.c. of a 10% suspension of washed human red blood corpuscles, 1 unit of amboceptor, its respective amount of dog serum, and physiologic salt solution to make the volume 1 c.c. These tubes were then incubated in a water bath at 37.5 C. for 30 minutes and examined. They were next placed in the ice box over night and in the morning read again. The least amount of dog serum bringing about complete hemolysis was considered the titer of the dog complement.

In titrating dog serum varying amounts

of a 1:10 dilution, as well as varying amounts of straight serum, were used. Inasmuch as 0.02 c.c. of guinea-pig complement was the unit for comparison, if it was found that 0.1 c.c. of straight dog serum was the titer of the dog complement, then the latter would be one-fifth as strong as guinea-pig complement.

In working with antisheep amboceptor, 0.5 cc. of a 1:1000 dilution was found to be a unit; that is, it produced complete hemolysis in a tube containing 0.5 c.c. of a 5% suspension of washed sheep corpuscles, an excess of complement, and sufficient salt solution to make the final volume 2.5 c.c. Complement was then titrated against this unit of amboceptor.

In most of the work the antihuman hemolytic amboceptor only was used, as it was very satisfactory.

The titer of the dog complement was then determined for several days preceding and immediately before and after the operation. It was then titrated 2 1/2, 6, 16, and 24 hours after the operation and at frequent intervals thereafter until the dogs were killed and the liver removed for microscopical examination. Animals were killed and tissue was obtained for study 30, 60, and 90 days after Eck's

fistula had been established. Liver tissue from apparently normal dogs was obtained for comparison.

Table 1 records the results of the titration. The data on Dogs 7 and 8 are also shown in the form of curves in the chart.

Table 1

The Complement Titer in Eck Fistula Dogs

	Titer of Dog Complement in Terms of Guinea-Pig Complement										
Dog	Immediately Before Operation	Immediately After Operation	2½ Hrs. After Operation	6 Hrs. After Operation	16 Hrs. After Operation	24 Hrs. After Operation	30 Days After Operation	60 Days After Operation	90 Days After Operation	Anesthetic used	Remarks
1	1/8	....	....	....	....	1/5	1/5	....	.....	Ether	Posted 30 da.
2	1/5	....	....	....	....	1/5	1/8	....	....	Ether	Posted 30 da.
3	1/10	....	....	....	....	1/12	....	....	....	Ether	Killed by mistake
4	1/5	1/10	1/10	1/8	1/5	1/5	1/5	1/5	....	Ether	Posted 60 da.
5	1/10	1/15	1/12	1/10	1/10	1/8	1/5	1/8	1/5	Ether	Posted 90 da.
6	1/6	1/12	1/10	1/8	1/5	1/5	1/5	1/8	....	Ether	Still living
7	1/5	1/10	1/8	1/8	1/5	1/5	1/5	1/8	....	Ether	Still living
8	1/8	1/30	1/15	1/5	1/8	1/5	1/5	....	....	Chloroform	Died from ammonia poisonin
9	1/4	1/25	1/15	1/5	1/5	1/5	1/5	1/10	....	Chloroform	Still living

The titer of the complement of individual normal dogs has been observed to fluctuate from one-fourth to one-twelfth that of guinea-pig complement.

Microscopically the livers of the dogs examined at 30, 60, and 90 days after the establishment of Eck's fistula, disclosed a progressive fatty degeneration and infiltration with necrosis of the liver cells. There was no corresponding decrease in complement accompanying the degenerative processes in the liver. Whether there will occur a drop in complement when the liver has completely degenerated, remains to be determined. Dogs are being carried over until next year for that purpose.

Ether was used as an anesthetic in 7 of the operations. In the case of the first 3 dogs, data on the complement unfortunately were not obtained until 24 hours after the operation when it was back to normal. In the next 4 dogs, immediately following the operation there was a drop in complement of about one-half, the complement returning to normal within 16 hours. In the 2 dogs operated upon under chloroform anesthesia the drop was very much greater, the titer going down to approximately one-fourth to one-sixth of the titer before the operation and returning to normal within 6 hours after the operation.

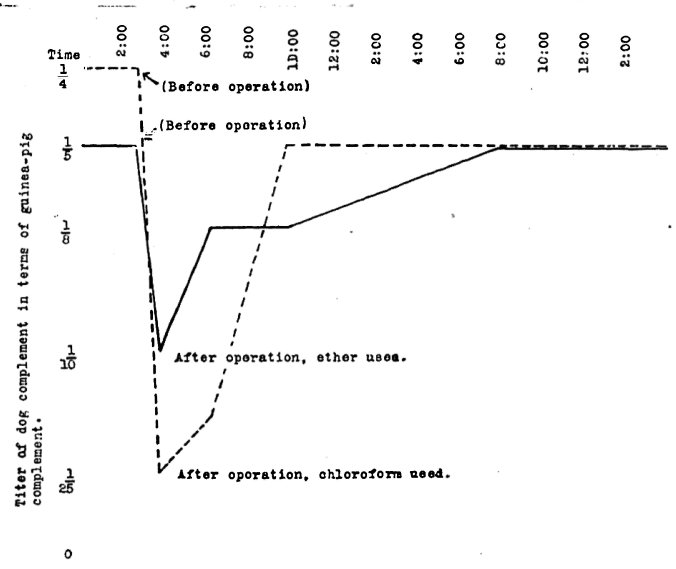


Chart 1. The broken line shows the titer of complement before and after the establishment of Eck's fistula when chloroform was used as the anesthetic. The solid line shows the titer of complement before and after the establishment of Eck's fistula when ether was used as the anesthetic.

We have here several factors all or part of which may contribute to the temporary drop in complement: (1) the interference with the normal function of the liver, (2) the sudden shunting of the portal circulation into the general circulation, (3) the effect of the anesthetic on the various tissue cells, leading not only to disturbed function but to change of blood constituents, (4) the presence of the anesthetic in the circulating blood in contact with the complement, and (5) the shock of the surgical operation. In view of the fact that the drop in complement was much less under ether

than it was under chloroform anesthesia, it would appear that the 3rd and 4th factors may be largely responsible for the immediate fall in complement.

In their work on Eck-fistula dogs, Matthews and Miller[8] emphasized the danger of the formation of adhesions following the operation, and the establishment of a collateral circulation for the liver. In the work here recorded great care was exercised to avoid this possibility. Adhesions were looked for carefully post mortem and data on dogs showing adhesions and collateral circulation were not included in the report. A few weeks after the operations the feces of the dogs became grayish-white in color, indicating the absence of bile. Symptoms of ammonia poisoning, as pointed out by Hahn, Massen, Nenchi, and Pawlow,[9] and Matthews and Miller,[8] could be easily induced by increasing the amount of meat in the diet. These were fair indications that a collateral circulation had not been established. In a few cases hemolytic substances appeared in the blood, but these could be eliminated easily by putting the dogs on a bread and water or milk diet for a couple of days. The complement was usually at a low normal, that is, one-eighth to one-twelfth that of guinea-pig serum, but normal dogs showed a corresponding drop

in complement when put on the same diet.

In regard to tissue changes in the liver following the Eck-fistula operation, Matthews and Miller make the following statement: "It is well known that after an Eck's fistula the liver soon begins to undergo fatty necrosis, which eventually invades the whole organ. This change in the nutrition of the organ may be responsible, in large measure, for the changes in metabolism so noticeable after an Eck's fistula." Macroscopically the liver showed a progressive atrophy. The rate of degeneration varied with different dogs, but was usually almost at a maximum in about 90 days. However, Dog 5 had not reached that stage at 90 days. In Dog 9, which at the writing of this report had been carried along 70 days, the liver was not palpable. The complement content on the 70th day was one-tenth that of guinea-pig serum. This dog, as well as normal dogs, had been kept off meat and fed on table scraps for 4 days previousto the titration. The sera of the normal dogs showed a complement content of one-tenth to one-twelfth that of guinea-pig serum at this time; previous to the change of diet it had been one-fifth.

CONCLUSIONS

The complement content of normal dog serum may vary in the same animal from one-fourth to one-twelfth that of guinea-pig serum.

Immediately following the establishment of Eck's fistula there is a temporary drop in complement lasting not more than 16 hours as a rule.

There is a much greater drop in complement under chloroform anesthesia than under ether anesthesia. The return to normal is apparently just as rapid when only sufficient chloroform is used to produce the surgical anesthesia.

The complement content of dog serum following the establishment of Eck's fistula was normal within from 6 to 16hours after the operation and remained normal for at least 90 days (last observation) in the dogs studied.

There is no corresponding drop in complement paralleling or accompanying the degeneration of the liver.

There does not seem to be sufficient evidence, as yet, to warrant the assumption that the liver plays a more important role in the production of complement than do other organs or tissues of the body.

BIBLIOGRAPHY

1. Gesammelte arbeiten zur Immunitatsforschung, 1904.
2. Bull. de l'Arcad. Roy. de Belg., 1908, Classe des Sc., p. 748. For a more complete bibliography of the work done on complement, see Zinsser's Infection and Resistance, 1914, and also the article by Dick.[5]
3. Centralbl. f. Bakteriol., I, O., 1911, 57, p. 577.
4. Weicharts Jahresbericht, 1912.
5. Jour. Infect. Dis., 1913, 12, p. 111.
6. Jour. Exper. Med., 1909, 11, p. 344.
7. Serum Diagnosis of Syphilis and the Butyric Acid Test for Syphilis, 1910.
8. Jour. Biol. Chem., 1913, 15, p. 87.
9. Arch. f. Exper. Path. u. Pharm. 1893, 32, p. 161.

STUDIES II

THE EFFECT OF VARIOUS CHEMICAL SUBSTANCES ON THE HEMOLYTIC REACTION

Hektoen[1] in 1903 pointed out that some of the inorganic salts, such as $CaCl_2$, $MgCl_2$, $BaCl_2$, $NaSO_4$, $Na_2C_2O_4$, and $Na_2C_2H_4O_6$, inhibit the hemolytic reaction when present in certain concentrations. This work was extended by Manwaring[2] and by Hektoen and Ruediger[3] to cover bacteriolysins, as well as hemolysins, and a much larger number of salts. They found that small doses of M/8 solutions of many salts prevent the lysis of red corpuscles and of bacteria by various sera, and concluded that the antilytic action is the result of the action of these salts on the complements. Arkin[4] (1913), in reporting on the effect of several substances on opsonins, mentions that lactic acid in certain concentrations not only inhibits phagocytosis, but prevents complement from combining with amboceptor in the hemolytic reaction. I have noted in a previous report[5] that a temporary drop in complement occurs during the Eck-fistula operation, which seems to be the result of the anesthesia. It is generally known that an acidosis usually results from the administration of ether or chloroform,[6] especially

following chloroform anesthesia, and perhaps this acidosis is a factor in causing the temporary drop in complement during Eck-fistula operations, inasmuch as the drop is much greater under chloroform than under ether anesthesia. Wendelstadt[7] showed that in the presence of a small amount of amboceptor glycogen suspends hemolysis by acting on the complement. Hektoen and Ruediger mention the work of Camus and Pagniez,[8] Neisser and Doehring,[9] and others, showing the ability of uremic serum to inhibit hemolysis by normal serum. Walker[10] and others have reported the presence of complement-fixing substances in the serum of a certain percentage of diabetics. Cumming[11] has shown that the sign of the Wassermann reaction may be changed by the influence of various salts, acids, and alkalies on the hemolytic system.

The work of this paper is an investigation of the effect of lactic acid, hydrochloric acid, sodium bicarbonate, and acetone on the complement content of rabbits in vivo; of ether and chloroform anesthesia on the hydrogen-ion concentration and complement content of the blood of normal rabbits; and lastly of carbon dioxide, lactic acid, hydrochloric acid, uric acid, urea, benzoic acid, tartaric acid, hydrazin sulfate, acetic acid, acetone, ether

and chloroform on the hemolytic reaction in vitro. It is hoped to extend this work to cover many other substances such as aceto-acetic acid, beta oxybutyric acid, etc., which are found under pathologic conditions, and also perhaps to cover some of the amino-acids. The group under discussion are also being tried out with reference to their effect on anti-body-production and on hemagglutinins and bacterial agglutinins.

EFFECT OF ETHER AND CHLOROFORM ANESTHESIA ON RABBIT COMPLEMENT IN VIVO.

Three series of rabbits were selected and the C_H^+ and the complement titer determined for each rabbit. The animals in Series 1 were then anesthetized with ether for varying lengths of time and the C_H^+ and complement content of their sera again determined. The animals in Series 2 were saturated with sodium bicarbonate by intravenous, subcutaneous, and intraperitoneal injections of a 2% solution of sodium bicarbonate, and the C_H^+ and complement content of their sera determined. The animals were then put under ether anesthesia for varying lengths of time, and the C_H^+ and complement content again determined. Series 3 was given chloro-

form for varying lengths of time, and data obtained as before. The results were as follows:

The hydrogen-ion concentrations[12] were determined by the colorometric method,[13] the gas-chain method not being available. To 1 c.c. of boiled double distilled water in a hemolytic test tube was added 1 drop of the indicator, the tubes were shaken, then 0.1 c.c. of the serum was added, and the tubes were again shaken, and read.

Since a hydrogen-ion concentration (C_{H^+}) of 10^{-7} is a truly neutral solution--that is the concentration of the hydroxyl ions (C_{OH}) is 10^{-7}--it will be noticed that rabbit blood seems to be slightly alkaline (10^{-8}) or neutral (10^{-7}) normally. Following ether anesthesia there is an increase in acidity. A drop in complement seemed to occur only when this increase in acidity passed the neutral point. In Series 2 the injection of sufficient sodium bicarbonate to change the C_{H^+} from 10^{-7} to 10^{-10} did not cause any variation in the complement content. Neither did the complement titer drop when thoseanimals which had been saturated with sodium bicarbonate were given ether anesthesia for varying lengths of time. The hydrogen-ion concentration did not return much past the neutral point of 10^{-7}. It is suggestive that Rabbit 5 had very little complement and a C_{H^+} of 10^{-6}; after the

animal had been saturated with sodium bicarbonate, the complement content returned to normal and the C_H^+ dropped to 10^{-8}. It has been stated that morphin prevents acidosis. Rabbit 3, Series 2, which received 1/12 gr. of morphin and then ether anesthesia, showed only a slight drop in complement content; the C_H^+ remained on the alkaline side of 10^{-7}, being 10^{-8}. These experiments, while not conclusive, are suggestive.

Table 1

SERIES 1, ETHER ANESTHESIA

Rabbit	C_H^+	Complement Titer, Undiluted, Serum (c.c.)	Time of Anesthesia (min.)	C_H^+	Complement Titer, Undiluted Serum (c.c.)
1	10^{-8}	0.05	10	10^{-6}	0.10
2	10^{-9}	0.03	15	10^{-7}	0.03
3	10^{-8}	0.03	20	10^{-6}	0.15
4	10^{-8}	0.08	45	10^{-5}	0.25

Table 2

SERIES 2, SODIUM BICARBONATE AND ETHER ANESTHESIA

Rabbit	C_H^+	Complement Titer (c.c.)	Material Injected (Sodium bicarbonate)	C_H^+	Complement Titer (c.c.)	Time of anesthesia (min.)	C_H^+	Complement Titer (c.c.)
1	10^{-7}	0.05	20 c.c. 2% sol.	10^{-10}	0.05	30	10^{-7}	0.05
2	10^{-7}	0.03	20 c.c.	10^{-9}	0.03	45	10^{-65}	0.08
3	10^{-7}	0.03	1/12 gr. morphin	10^{-8}	0.03	15	10^{-8}	0.05
4	10^{-7}	0.05	40 c.c.	10^{-10}	0.05	30	10^{-7}	0.05
5	10^{-6}	0.30	30 c.c.	10^{-8}	0.05	15	10^{-7}	0.05

Table 3

SERIES 3, CHLOROFORM

Rabbit	C_H^+	Complement Titer (cc.)	Time of Anesthesia (min.)	C_H^+	Complement Titer (cc.)
1	10^{-8}	0.03	10	10^{-7}	0.03
2	10^{-8}	0.03	30	10^{-5}	0.40
3	10^{-8}	0.05	15		Killed
4	10^{-7}	0.05			Killed

EFFECT OF LACTIC ACID AND HYDROCHLORIC ACID ON COMPLEMENT IN VIVO

For this purpose 4 rabbits were selected, 2 for treatment with lactic acid and 2 for treatment with hydrochloric acid. The complement content and C_H^+ for each were determined and then 2 received each the equivalent of 0.5 c.c. of N/1 lactic acid intravenously, and 1 c.c. intraperitoneally, and the other 2 received each corresponding amounts and equivalents of N/1 hydrochloric acid. The C_H^+ and complement content were then determined and greater amounts of material injected. The results were as given in Table 4.

Table 4

Data on Rabbits Injected with Lactic Acid

Rabbit	C_H^+	Complement Titer (cc.)	Time of of Lactic Acid Injected(cc.)	C_H^+	Complement Titer(cc.)
1	10^{-7}	0.10	1.5	10^{-7}	0.08
	10^{-7}	0.05	6.0	10^{-7}	0.05
	10^{-7}	0.05	4.0	10^{-7}	0.05
2	10^{-7}	0.08	1.5	10^{-7}	0.05
	10^{-7}	0.05	6.0	10^{-7}	0.05
	10^{-7}	0.05	4.0	10^{-7}	0.05

The injection of lactic acid did not cause a drop in complement; it rather favored an increase in the complement content. The fact that the body is able to oxidize many of the organic acids, including lactic acid, over to the carbonates and that they then act as alkalies, would account for the similarity of action between lactic acid and sodium bicarbonate.

Twenty-four hours after these data were obtained, both animals received additional doses of lactic acid; death resulted. In administering the acid the desired amount of normal solution was pipetted out and diluted 15 or 20 times with isotonic salt solution, and this diluted solution injected.

The results of the action of hydrochloric acid are given in Table 5.

Table 5

DATA ON RABBITS INJECTED WITH HCl

Rabbit	C_{H^+}	Complement Titer(cc)	Material and Amt. injected	C_{H^+}	Complement Titer (cc.)	Remarks
1	10^{-8}	0.03	5 cc.N/20 HCl	10^{-65}	0.10	
	10^{-65}	0.10	15 cc.N/20 HCl	10^{-6}	0.15	
	10^{-6}	0.15	20 cc.2% Na_2HCO_3			Died
2	10^{-8}	0.05	5 cc.N/20 HCl	10^{-7}	0.10	
	10^{-7}	0.10	15 cc.N/20 HCl	10^{-6}	0.15	
	10^{-6}	0.15	20 cc.2% Na_2HCO_3			Died

The results are in keeping with the fact that the body is unable to oxidize the mineral acids. The drop in complement did not occur immediately, but several hours after the administration of the acid. Normal variation in the rabbit complement might also account for a part, at least, of the variation. The complement might be affected also by any CO_2 liberated as the result of injection of dilute HCl and its action on the carbonates present in the body. Accordingly, fresh complement was taken and CO_2 bubbled through it for varying lengths of time up to 15 minutes. The hemolytic reaction was inhibited as long as CO_2 was present in an appreciable amount, but proceeded as soon as the CO_2 was driven off.

Since in a typical acidosis, such as one finds in diabetes, aceto-acetic acid, beta oxybutyric acid, acetone, and CO_2 are increased in the blood stream, it was next decided to try out acetone, aceto-acetic acid and beta oxybutyric acids not being available. Two rabbits were each given an injection of a 2% aqueous solution of acetone every hour for several hours, 20 c.c. being given at each injection. The C_{H^+} and complement content were determined before and after each injection and on thehalf hour between. The C_{H^+} remained from the beginning at 10^{-8} for Rabbit 1

and at 10^{-7} for Rabbit 2, and the complement titer did not vary from the original normals; that is, 0.03 c.c. contained 1 unit in Rabbit1and 0.05 c.c., 1 unit in Rabbit 2. Quantitative determinations of the amount of acetone in the blood stream or urine were not made. Again, these results might be explained on the basis of a not sufficient concentration of acetone in the blood stream, as well as that acetone by itself has no effect on complement in vivo. The former is a plausible explanation in view of the work of Manwaring, Hektoen, and others on the antilytic action of various salts when the concentrations were found to be important in vitro.

EFFECT OF DIFFERENT CONCENTRATIONS OF THE REAGENTS ON THE HEMOLYTIC REACTION

It was next decided to determine the effect of different concentrations of lactic acid, hydrochloric acid, uric acid, benzoic acid, tartaric acid, urea, hydrazin sulfate, acetic acid, acetone, ether, and chloroform on the hemolytic reaction.

In doing this it was thought necessary to determine the effect, if any, of each of these reagents on red blood cells, complement, and amboceptor, respectively. In working with lactic and hydrochloric

acids, normal and decinormal solutions were carefully made up and standardized, and, when necessary, dilutions of these were carefully made. In the case of uric acid, benzoic acid, and hydrazin sulfate, on account of their slight solubilities, saturated solutions at 37 C. were used. It would perhaps have been better to have made up fractional parts of their molecular solutions based on their solubilities. In dealing with urea and tartaric acid, M/1 and M/10 solutions were carefully made in isotonic salt solution. The antihuman hemolytic system was used, with rabbit complement. Titrations of amboceptor and complement were made daily. One unit of amboceptor and 1 unit of complement were used in the tests for determining the effect of the reagents on amboceptor and complement respectively. Thirty-minute incubations at 37.5 C. in a water bath were used.

Various concentrations of each of the reagents were used in combination with red blood cells and salt solution to determine whether the substance was hemolytic, and if so, in what concentrations. Next, various concentrations were added to tubes containing salt solution and 1 unit of amboceptor, and the whole incubated 30 minutes at 37.5 C. in a water bath. Several volumes of salt solution were then added to bring the concentration below that which of itself would produce hemolysis, red blood cells

were added, and the tubes shaken and again incubated to permit of the union of amboceptor and blood cells. The tubes were then removed and centrifugated, and the cells washed several times with salt solution. Enough salt solution plus 1 unit of complement was then added to bring the volume to 1 cc., and the tubes were replaced in the water bath. In no case was there observed any effect exerted on amboceptor by any of the reagents used.

In testing the effect on complement 1 unit of complement was placed in each test tube along with salt solution, various concentrations of the reagent under investigation added, and the whole incubated 30 minutes. Red blood cells and amboceptor were then added and the tubes re-incubated. Controls were made in which the red blood cells and amboceptor had been permitted to unite before adding the reagents, and also controls were made for the hemolytic system. Data on the hemolytic activity had already been obtained. In the following tables varying degrees of hemolysis are indicated by the symbols used in the Wassermann reaction; a minus sign indicates complete hemolysis, and 4 plus signs indicate absence of hemolysis.

All the reagents included in Table 6, with the exception of uric acid and urea, were able

to bring about laking of human red blood cells. Uric acid is a very weak acid, and this together with its very slight solubility may be important factors in eliminating it from the group of hemolyzers. Urea, while comparatively soluble, is slightly basic in reaction, and apparently does not have an affinity for either the stroma or the hemoglobin of the cell. The group of hemolytic substances mentioned in this table contains mineral acids, fatty acids, aromatic acids, monohydroxy and dihydroxy acids, acetone, ether, chloroform, and hydrazin sulfate.

TABLE 6

EFFECT OF THE VARIOUS REAGENTS ON HUMAN BLOOD CELLS

REAGENT	Amount (cc) used and results											
	1	2	3	4	5	6	7	8	9	10	11	12
N/25 lactic acid	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 +++	0.09 ++++	0.08 ++++	0.05 ++++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
N/30 HCl.....	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 ---	0.08 ----	0.05 ----	0.03 ----	0.02 +++	0.01 ++++	0.00 ++++	
Saturated solution of uric acid.	0.8 ++++	0.7 ++++	0.6 ++++	0.5 ++++	0.4 ++++	0.3 ++++	0.2 ++++	0.1 ++++	0.08 ++++	0.05 ++++	0.03 ++++	0.00 ++++
M/1 solution of urea...	0.8 ++++	0.7 ++++	0.6 ++++	0.5 ++++	0.4 ++++	0.3 ++++	0.2 ++++	0.1 ++++	0.08 ++++	0.05 ++++	0.02 ++++	0.00 ++++
Saturated solution of benzoic acid	0.3 ---	0.2 ---	0.1 ---	0.09 ----	0.08 ----	0.06 ----	0.04 ----	0.03 +	0.02 ++++	0.01 ++++	0.00 ++++	
M/10 tartaric acid	0.2 ---	0.1 ---	0.09 ----	0.08 ----	0.07 ----	0.06 +	0.05 ++	0.04 +++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
1:10 dilution of saturated solution of hydrazin sulfate.....	0.4 ---	0.3 ---	0.2 ---	0.1 ---	0.08 ---	0.05 ---	0.04 ---	0.03 +	0.02 ++++	0.01 ++++	0.00 ++++	
M/10 acetic acid.....	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 ±	0.08 +	0.06 ++	0.04 +++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++
Acetone, undiluted.....	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 ++++	0.08 ++++	0.05 ++++	0.04 ++++	0.02 ++++	0.01 ++++	0.00 ++++	
Ether	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 ---	0.08 +	0.05 ++++	0.03 ++++	0.02 ++++	0.01 ++++	0.00 ++++	
Chloroform*.....	0.5 ---	0.4 ---	0.3 ---	0.2 ---	0.1 ---	0.08 ---	0.05 ---	0.03 ++	0.02 +++	0.01 ++++	0.00 ++++	

Even tubes 8, 9, and 10 have an excess of chloroform which would eventually hemolyze the blood cells For accurate quantitative work on red blood cells and complement less than these amounts should be used. Blood cells from the author were not hemolyzed in the time mentioned; cells from another individual in the laboratory were.

TABLE 7

EFFECT OF THE VARIOUS REAGENTS ON COMPLEMENT

Reagent	Amount(cc) Used and Results											
	1	2	3	4	5	6	7	8	9	10	11	12
N/25 lactic acid.....	0.5 -	0.4 -	0.3 +++	0.25 ++++	0.2 ++++	0.15 +++	0.1 + -	0.08 -	0.06 -	0.04 -	0.02 -	0.00 -
N/30 HCl.....	0.4 -	0.3 -	0.2 -	0.15 ++	0.10 ++++	0.09 ++++	0.08 ++++	0.07 +++	0.05 -	0.03 -	0.01 -	0.00 -
Saturated solution of uric acid	0.8 -	0.7 -	0.6 -	0.5 -	0.4 -	0.3 -	0.2 -	0.1 -	0.08 -	0.05 -	0.02 -	0.00 -
M/1 urea :.....	0.5 ++++	0.4 ++++	0.3 ++++	0.2 +++	0.1 -	0.08 -	0.06 -	0.03 -	0.01 -	0.00 -		
Saturated solution of benzoic acid....	0.4 -	0.3 -	0.2 -	0.1 ++++	0.09 ++++	0.08 ++++	0.07 ++++	0.06 -	0.05 -	0.04 -	0.02 -	0.00 -
M/10 tartaric acid	0.4 -	0.3 -	0.2 -	0.1 -	0.08 +++	0.06 ++++	0.05 ++++	0.04 ++++	0.02 ++	0.01 -	0.00 -	
1:10 dilution of saturated solution of hydrazin sulfate..	0.4 -	0.3 -	0.2 -	0.15 +	0.1 ++++	0.09 ++++	0.08 ++++	0.07 +++	0.05 -	0.03 -	0.01 -	0.00 -
M/10 glacial acetic acid	0.5 -	0.4 -	0.3 -	0.2 +	0.1 ++++	0.08 ++++	0.06 ++++	0.04 ++++	0.02 +	0.01 -	0.00 -	
Acetone, undiluted	0.2 -	0.15 +	0.10 ++++	0.09 ++++	0.08 ++++	0.07 +++	0.06 +	0.05 -	0.03 -	0.02 -	0.01 -	0.00 -
Ether	0.05 ++++	0.04 ++++	0.03 ++++	0.02 +++	0.01 +	0.00 -						
Chloroform	0.03 ++++	0.02 ++++	0.01 ++++	0.00 -								

DISCUSSION

Little is known as to the exact mechanism of hemolysis. Moore[13] and others, in working with fatty acids and soaps, arrived at the general conclusion that hemolytic agents depend on the presence of a double bond uniting 2 carbon atoms. While this might hold true for the unsaturated fatty acids and soaps, the reagents under discussion in this paper do not fit in with his observations. Matthews,[14] Lilly,[15] and others, mention that ether and chloroform are hemolytic agents, and it has long been known that mineral acids would act accordingly. So far as known, no report has been made on the remaining substances mentioned in this paper.

Mathews[16] has very clearly summed up the more recent views held as to the way hemolytic agents bring about their results. He states:

"Hemoglobin may be held in the corpuscle by union with the stroma. It is true for all other cells, and probably it is true for the corpuscles, that they are not bags filled with fluid, but they are organized jellies. The corpuscles behave in many ways as if they also were such jellies. Hemoglobin does not escape as one would expect it would

if it were in solution, when the corpuscle is punctured or cut across, but it stays in the divided corpuscle. Moreover, when hemoglobin is set free in the corpuscle by some of these methods, particularly in the very large cells of Necturus, a tailed amphibian, the hemoglobin may crystallize in the corpuscle itself, which shows that it must be prevented in some way from crystallizing in the normal cell. Moreover, the concentration of hemoglobin in the mammalian corpuscle is greater than the solubility of oxyhemoglobin in an equal bulk of water. For these and other reasons some observers are of the opinion that hemoglobin is held in some kind of a loose chemical or physical union, presumably the former, with the stroma of the corpuscles and that the various hemolytic agents break this union. It is not at all impossible that the union is with certain reserve valencies of the hemoglobin and the stroma and such unions are very unstable and easily broken."

It is also quite probable that all the reagents do not act in exactly the same way. The color of the supernatant fluid resulting from the action of oxidizing agents, such as the acids, on the red blood cells is greenish probably because of the predominance of biliverdin, whereas the color follow-

ing hemolysis by chloroform is more nearly a cherry red, similar to that following the action of immune sera.

It might be worthy of note in discussing the reagents used in this work that the lactic acid was of the racemic form, as is all lactic acid produced synthetically. This differs from the sarcolactic acid found in the body in that is is composed of both d-lactic and l-lactic acid, whereas the latter is d-lactic acid only. There might be some difference, but it is probable, in view of the general hemolytic properties of acids, that it would be a quantitative one only. The racemic form of lactic acid is found in the intestinal tract, and under pathologic conditions, may be present in the stomach. Sarcolactic acid is normally present in the tissues of the body and is especially increased during fatigue. The other hydroxyacid, tartaric, is dihydroxysuccinic acid, found in wine and some fruits, which are probably the source of this acid. Benzoic acid, an aromatic acid, is also taken into the body in fruits and berries. Bacterial decomposition may lead to the formation of phenyl carbonic acid (benzoic acid) from the proteins by a series of oxidation reactions. Acetic acid, a fatty acid, is

also produced in the intestinal tract as a result of bacterial fermentation. In a typical acidosis there are present aceto-acetic acid, acetone, and b-oxybutyric acid.

Hydrazin sulfate was studied because it was used by Dick[17] to produce necrosis of liver cells in dogs under observation for complement content. This probably breaks up into hydrazin and sulfuric acid. It is quite possible that hemolysis may result from the hydrazin sulfate molecule, as well as from the action of the dissociation products.

None of these reagents is as strongly hemolytic as the unsaturated fatty acids, which will act in dilutions many times greater than any of the dilutions in which the other reagents mentioned are active.

One point in addition as to the mode of action of these hemolytic agents, is that, if the red blood cells are colloidal jellies, contact with free hydrogen ions will permit of a somewhat greater intake of water, and this, according to Brahmachari,[18] materially increases the amount of hemolysis.

An inspection of the table showing the effect of the various reagents on complement shows

that all except uric acid were capable of preventing hemolysis. Sachs and Altman[19] (1908), working with unsaturated fatty acids and soaps, showed that these were able to prevent hemolysis by hemolytic sera, and concluded that the action was that of an anticomplement. Moore, Wilson, and Hutchinson, in reviewing this work, speak of the action as a balancing action of hemolyzers; they do not believe that these reagents act as anti-complements. They say in part:

"This result has been stated to be due to the neutralizing of complement, the sodium oleate acting as an anti-complement. We think, however, that there is clear evidence against this view. In the first place, as we shall see later, an ordinary serum which is not hemolytic to the corpuscles being used, it may, in fact, be their own serum, is strongly protective against the hemolytic action of sodium oleate. We have followed this question up in detail, as shown by the protocols of our experiments and have successively removed or destroyed (a) immune body, (b) complement, and (c) the lipoids from the active serum. In all cases we have found that no one of these substances is alone responsible for the neutralizing of the hemolytic activity of the sodium oleate. That is to say, in the active serum the immune body and the sodium oleate or

linoleate combine and mutually destroy each other's laking power, so that the mixture in due proportion is quite inert upon the blood corpuscles."

The importance of this citation will be evident upon a close inspection of Tables 6 and 7. In the first place urea, which is not a hemolyzer, nevertheless prevents hemolysis by amboceptor and complement. This cannot be a balancing action of hemolyzers. Moreover, of the hemolytic agents which fix complement, the zone of fixation very closely approximates, and in many cases partially coincides with, the weakest dilutions producing hemolysis, and in many cases extends down to include a few which are unable to produce hemolysis. This is a phenomenon very similar to the one mentioned by Moore, Wilson, and Hutchinson. They do not seem to have taken into consideration the fact that the "ordinary serum" they mention as inhibiting the action of their hemolytic agent contained complement. They intimate that complement did take some part in the inhibiting action. After careful investigation of the substances mentioned in Tables 6 and 7, it was finally decided that, while other colloids present in fresh normal serum might take some part, the themolabil colloid called complement played a large part. Whether in the nonhemolytic concentrations that fixed comple-

ment the effect was on the midpiece or endpiece of complement, was not investigated. It is a well-known fact that in the Wassermann reaction the midpiece alone is bound, the endpiece remaining free, in the supernatant fluid.

I have shown also that CO_2 prevents complement from combining with amboceptor, but does not prevent amboceptor from combining with the red blood cell. This confirms the work of Sawtschenko,[20] who showed that in reality the midpiece unites with amboceptor, but that the endpiece is unable to unite with the midpiece. As soon as the CO_2 is removed, the reaction occurs. As Mathews says, this is probably one of the means the body has of preventing hemolysis in vivo by hemolytic sera.

Hektoen and Ruediger's work with the inorganic salts showed that these salts act as anti-complements in which the ions of Ca and Ba, etc., combine with the complement, giving Ca-complement, Ba-complement, etc.

The molecule of ether is not broken up in the animal body, as is that of chloroform. Graham[21] showed that in the blood stream chloroform ($CHCl_3$) is broken up into $COCl_2$ (Phosgene) and HCl. It would then appear that there are several possibilities for the

fixing of complement by chloroform, as all these would possibly be present in the serum removed from an anesthetized animal.

The work of Lillie,[15] McClendon,[22] and others, is of interest because it suggests that the anesthetics may, in certain concentrations, play a double role in preventing hemolysis by amboceptor and complement. Lillie has observed that anesthetics prevent the outward diffusion of cell pigment produced by salts and thinks this a direct proof that anesthetics prevent increase in cell permeability. In order to determine positively whether the anesthetics were able to destroy or deflect complement in addition to this effect on the cell membrane, the following experiment was made:

One unit of complement (0.1 c.c.), 0.65 c.c. of physiologic salt solution, and 0.05 c.c. of ether were added to each of 3 hemolytic test tubes. These tubes were incubated 15 minutes at 37.5 C. and 0.1 c.c. of a 10% suspension of washed human red blood cells and 1 unit (0.1 c.c.) of antihuman hemolytic amboceptor added. The tubes were again incubated for 15 minutes in the water bath, centrifugated, and the supernatant fluids from all 3 tubes pipetted into a shallow dish, over which a current of air was passed

to evaporate the ether. The centrifugated red blood cells in each of the three tubes were washed repeatedly with isotonic salt solution. After the last washing the supernatant fluids were removed and washed; 0.9 c.c. of physiologic salt solution was then added to Tube 1, and 0.8 c.c. to Tube 2 together with 1 unit of complement; to Tube 3 was added 0.9 c.c. of the original supernatant fluid from which the ether had been evaporated. A 4th tube was then placed in the rack and to it were added 0.1 c.c. of a 10% suspension of washed human red blood cells and 0.9 c.c. of supernatant fluid similar to that added to Tube 3. The tubes were then incubated at 37.5 C. in a water bath for 30 minutes.

The results were no hemolysis in Tubes 1 and 4, and complete hemolysis in Tubes 2 and 3. Hence it is evident that amboceptor had combined with the red blood cells in the presence of the anesthetic, and that all the complement had been deflected but not destroyed. In a future paper a report will be made as to the action of the various reagents on endpiece, midpiece, and the so-called third component of complement mentioned by Ehrlich,[23] Coca,[24] and others. These results are of interest in view of the inhibiting effect of ether on phagocytosis in vitro and the lowered

resistance following anesthesia as shown by Graham.[25] He found that the opsonic power of an etherized serum was restored by evaporating the ether. My work would suggest as an explanation of his results that the ether prevented the complement from combining with the opsonic immune body. The negative results which he obtained for bacteriolysins are not comparable, owing to the necessity of using lower concentrations of ether in these experiments than in those on phagocytosis. They do suggest that in the animals body it is necessary to take into consideration other factors such as acidosis, etc., in addition to the anesthetic itself in order to account for all of the lowered resistance.

If data were available it would be interesting to tabulate for the various secretions, excretions, body fluids, and organ content the amount, if any, of each of these and other reagents present normally, as well as the amount under pathologic conditions, and compare these with the amount necessary to deflect or destroy complement. This might throw light on some acute and chronic infections, as well as on some focal infections. For example, normally the urine contains from 0.005 gm. to 0.02 gm. of urea per cubic centimeter; from Table 2 it may be shown that 0.012 gm. of urea is enough to fix 1 unit of complement.

These data might be an explanation of the discrepancy of reports as to the presence or absence of complement in normal urine. It is conceivable that a cystitis might become chronic as a result of the presence of chemical substances interfering with phagocytosis. More recently[26] it has been shown that urea is increased in the blood during typhoid fever, especially in the severe cases. Mathews says that under certain pathologic conditions, such as interference with kidney function, the urea may increase to such an extent as to crystallize out on the skin on evaporation of perspiration. Urea may also be increased materially in the saliva, and particularly in the secretions of the duodenum and of the intestine.

Uric acid under normal conditions is present in about 0.00003 gm. per cubic centimeter of blood; under pathologic conditions this may be increased to 0.00015 gm. per cubic centimeter of blood. The amount present in the maximal amount of saturated solution used was only 0.000025 gm., which is one-sixth the amount which may be present under pathologic conditions. Hence, while complement is not affected by the amount used, it may well be that complement is deflected or destroyed by the same amount present under

certain pathologic conditions.

From these data it is also clear how hyperacidity of the stomach may favor a chronic infection of the stomach. The same may be true for the intestinal tract in general.

It is also conceivable that an accumulation of by-products of metabolism in any organ or tissue may similarly lower the resistance of that organ or tissue so as to lead to a localization of infection there. On the other hand, it was noticed that many of the reagents studied seemed to hasten the hemolytic reaction when the concentrations used were much less than those fixing complement; that is, in many cases these tubes hemolyzed before the controls on the hemolytic system did. Hence it is conceivable that in certain weak concentrations many of these organic compounds may increase the efficiency of the immunity mechanism of the body, while in greater concentrations they may act in the reverse manner. It would be well worth while to work out the effect of as many of these reagents as possible on bacteriolysins, opsonins, etc., and also to determine whether it is possible to get a summation of effects, or whether anything acts as a protective substance, interfering in the body with the possible harmful effect of one or more of these compounds.

SUMMARY AND CONCLUSIONS

An apparent drop in complement often occurs as a result of anesthesia. This, however, is not constant.

The apparent drop probably is the result of the presence of the anesthetic, as well as of a slight increase in acidity.

Sodium bicarbonate and lactic acid injected into rabbits did not cause a drop in complement; if anything, they caused an increase. The lactic acid was probably oxidized over into carbonates by the body, thereafter acting as an alkali.

HCl injected into rabbits caused a marked drop in complement. This is as might be expected, since the body is unable to oxidize the mineral acids.

Acetone injected in relatively large amounts failed to cause a drop in complement.

Carbon dioxid, lactic acid, HCl, urea, benzoic acid, tartaric acid, acetic acid, acetone, ether, chloroform, and hydrazin sulfate deflect or destroy hemolytic complement in certain concentrations.

All these reagents, except urea and CO_2, bring about laking of human red blood cells in certain concentrations.

In general it may be said that the zone

of concentrations fixing complement very nearly approximates, and in many cases coincides with, the weakest dilutions producing hemolysis, and may extend down to include a few concentrations which are unable to produce hemolysis.

The amount present in the blood is normally many times less than the amount required to affect complement. Under some pathologic conditions the concentration in the blood may be greatly increased, nearly approaching the amount which would affect complement.

On a rich protein diet the amount of urea normally in urine may exceed the amount necessary to fix complement.

This work suggests the possibility that the power of various organic compounds to deflect or fix complement may be a factor favoring infection or maintaining a chronic infection.

In addition to the selective action of many bacteria for certain tissue as the explanation of their localization therein (Rosenow), there may be also the fact of a lowered resistance of the organ or tissue due to increase of products of metabolism therein.

In very weak concentrations--that is, concentrations much too weak to fix complement--many of these reagents seem to accelerate the hemolytic reaction. This is analogous to the physiologic action of many pharmacologic products. Acting in this manner they might increase the efficiency of the immunity mechanism of the body.

At least in the case of the anesthetics there is a two-fold mechanism inhibiting hemolysis by amboceptor and complement--the action of the anesthetic on the permeability of the membrane, and the deflection of complement.

A saturated solution of uric acid in physiologic salt solution (37 C.)did not hemolyze red blood cells or fix complement. This concentration is however only one-sixth of that found under certain pathologic conditions.

None of these reagents in the concentrations studied prevented amboceptor from combining with red blood cells.

At least in the case of CO_2, ether, and chloroform, complement was not destroyed; but part or all of it was deflected.

This work suggests the possibility that some organic compounds might occasionally play some role

in either inhibiting or intensifying the anaphylactic shock, since complement seems to be involved in this reaction.

BIBLIOGRAPHY

1. Trans. Chicago Path. Soc., 1903, 5, p. 303.

2. Jour. Infect. Dis., 1904, 1, p. 112.

3. Ibed., p. 379

4 Ibid.,1913, 13, p. 408

5. Shereood, Smith, and West, Jour. Infect. Dis., 1916, 19, p. 682.

6. Woolley, Jour. Lab. and Clin. Med., 1916, 1, p. 782

7. Centralbl. f. Bakteriol., I. O., 1903, 34, p. 83.

8. Camua and Pagniez, Compt. rend. Soc. de biol., 1901, 53, p. 730

9. Berl. klin. Wchnschr., 1901, 38, p. 593.

10. Jour. Am. Med. Assn., 1916, 66, p. 488.

11. Jour. Infect. Dis., 1916, 18, p. 151.

12. Washburn, Proc. Illinois Water Supply Assn., 1910, p. 93.

13. Kelley, Jour. Lab. and Clin. Med., 1915, 1, p. 195.
Wilson and Hutchinson, Jour. Biol. Chem., 1909, 4, 346.

14. Phys. Chem., 1915, p. 924.

15. Jour. Exoer. Zool., 1914, 16, p. 591.

16. Phys. Chem., 1915, p. 497.

17. Jour. Infect. Dis., 1913, 12, p. 111.

18. Jour. Biol. Chem. 1909, 4, p. 280.

19. Berl. klin. Wchnschm., 1908, 10, p. 494.

20. Ann. de l'Inst. de Pasteur, 1912, 26, p. 1032.

21. Jour. Exper. Med. 1915, 22, p. 48.

BIBLIOGRAPHY (Cont'd)

22. Am. Jour. Physiol. 1915, 38, p. 173.

23. Studies in Immunity, 1906.

24. Ztschr. f. Immunitatsf., 1914, 21, p. 604.

25. Jour. Infect. Dis., 1911, 8, p. 147.

26. Jouve-Belmelle, Progres Med., 1916, 31, p. 149.
Abstracted Jour. Am. Med. Assn., 1916, 67, p. 1189.

STUDIES III

THE EFFECT OF UREA UPON IMMUNOLOGICAL REACTIONS

In a previous communication[1] I reported that urea and certain other organic compounds in certain concentrations would inhibit the action of rabbit complement, and suggested that it and other organic compounds might have more or less effect upon immunological reactions within the body as well as in test tube experiments. Symmers and Kirk[2] have reported the favorable action of urea in the treatment of war wounds. They note that in very strong concentrations urea had a pronounced germicidal action. Solis-Cohen, Kolmer and Heist[3] have reported upon the action of quinine and urea as well as various cinchona derivatives upon pneumococcus infections in mice. They report that urea in concentrations of 1-100 or higher did not possess any noticeable germicidal properties. From their work it would appear that quinine and urea was more efficient than quinine alone.

The present paper is a continuation of the work already reported upon with a view first to determining the effect of urea upon human, guinea pig, dog and hog complement and the effect upon normal dog anti-human hemolysins in test tube experiments. Second,

the effect of intravenous injection of known amounts of urea solution on rabbit complement compared with the intravenous inoculation of the same quantities and molecular strength of sodium chloride solution and these compered with the blood ureas determined from hour to hour . Third, to determine the effect of intravenous inoculation of the same amounts of urea solution and sodium chloride solution as in "2" upon the white blood count of normal rabbits. Fourth, to determine the effect of intravenous inoculation of urea on streptococcus infections in rabbits.

Throughout this work the anti-human hemolytic system was used. The technic employed in determining the data for "1" was similar to that described in a previous communication.[1] The results are summarized in Tables 1 and 2, end graphically illustrated in plate 1, fig. 1. The technic used in determining the effect on coaplement in vivo of M/1 urea and M/1 NaCl solutions was essentially as follows:

Blood urea determinations sere made several times to determine the normal urea content per 100 c.c. of blood. Immediately after the blood was drawn for the last determination 2 c.c. of M/1 urea was injected into the marginal ear vein of one rabbit

while 2 c.c. of M/1 NaCl was injected into the corresponding ear vein of another rabbit. The rest of the injections and urea determinations were carried on approximately every hour as shown in the subsequent tables. The quantitative determination of urea was essentially that given by Gradwohl[4] except that the ammonia was sucked over into a known amount of N/100 HCl and the amount of uncombined acid determined by direct titration using methyl orange as an indicator. From this the amount of urea per 100 c.c. of blood was calculated. These results are summarized in table 3 and graphically shown in plate 1, fig. 2. The data for "3", that is, the effect of the injection of molecular solutions of urea and NaCl upon the white blood count, is summerized in tables 4 and 5 and plate 2, figs. 1 and 2.

In studying the effect of urea upon streptococcus infections in rabbits, three groups of rabbits were selected; two adults weighing approximately 2700 grams, three half-grown rabbits weighing approximately 1500 grams each, and five young rabbits weighing approximately 600 grams each. Preliminary experiments were made to determine the fatal doseage in four days of a hemolytic streptococcus isolated

from a case of erysipelas. It was found that 10 cc. of a 24-hour calcium carbonate broth culture of this streptococcus injected daily until four injections had been given would uniformly kill in four days either adult or young rabbits. Accordingly this amount was used. In the first series of rabbits as mentioned above, one rabbit was picked from each group for treatment; that is, one of the two adults and one of the three rabbits which weighed approximately 1500 grams and one of the five young rabbits weighing approximately 600 grams. The word "treatment" is used advisedly as it will be brought out subsequently that this may not be the proper word to use. In this first series the urea was added to the broth suspension just before injection. The amounts of urea used were 0.15 grams for adult rabbits, 0.1 gram for those weighing approximately 1500 grams, and 0.1 gram for the young rabbits. A second set of experiments was carried out comparable to this set in which the broth culture was injected into one ear and the urea into the opposite ear. These results are summarized in the following tables.

TABLE 1
EFFECT OF UREA ON COMPLEMENTS OF DIFFERENT ANIMALS

Serum used for Complement CC	Tubes numbered from 1 to 10 showing amount (cc) of M/1 Urea and results										Remarks
	1	2	3	4	5	6	7	8	9	10	
	0.00	0.02	0.04	0.06	0.08	0.1	0.2	0.3	0.4	0.5	
Human											(Incubated 30" in
"J"	-	+	3+	4+	4+	4+	4+				(water bath and 2
"I"	-	+	3+	4+	4+	4+	4+				(hrs. in ice box.
"S"	-	+	2+	2+	4+	4+	4+				(
"SR"	-	+	3+	4+	4+	4+	4+				After 30" water bath
	-	-	2+	3+	4+	4+	4+				After standing 24 hrs
Dog											
#1	-	-	-	-	-	±	4+	4+	4+	4+	After 30"water bath
	-	-	-	-	-	-	2+	3+	4+	4+	After 2 hr ice box
#5	-	-	-	-	-	+	4+	4+	4+		After 30"water bath
	-	-	-	-	-	-	-	2+	4+		After 2 hrs.ice box
#6	-	-	-	-	-	±	3+	3+	4+	4+	After 30"water bath
	-	-	-	-	-	-	-	2+	4+		After 2 hrs.ice box
	-	-	-	-	-	-	-	-	-		After 24 hrs.icebox
Hog											
#1	-	-	-	-	±	3+	4+	4+	4+		After 30"water bath
	-	-	-	-	-	±	2+	3+	4+		After 2 hrs ice box
#2	-	-	-	-		4+	4+	4+	4+		After 30"water bath
	-	-	-	-	-	2+	3+	4+	4+		After 2 hrs.ice box

TABLE 1 - continued
EFFECT OF UREA ON COMPLEMENTS OF DIFFERENT ANIMALS

Serum used for Complement cc.	Tubes numbered from 1 to 10 showing amount (cc) of M/1 Urea and results										Remarks
	1	2	3	4	5	6	7	8	9	10	
	0.00	0.02	0.04	0.06	0.08	0.1	0.2	0.3	0.4	0.5	
Rabbit											
#1	-	-	-	-	-	2+	4+	4+	4+		After 30"water bath
	-	-	-	-	-	-	3+	4+	4+		After 2 hrs.ice box
#2	-	-	-	-	-	-	±	1+	4+	4+	After 30"water bath
	-	-	-	-	-	-	-	-	4+	4+	After 2 hrs ice box
	-	-	-	-	-	-	-	-	2+	2+	After 24 hrs icebox
#3	-	-	-	-	-	-	-	-	-	3+	After 30"water bath
	-	-	-	-	-	-	-	-	-	-	After 2 hrs ice box
#4	-	-	-	-	-	-	-	1+	3+	4+	After 30"water bath
	-	-	-	-	-	-	-	-	1+	4+	After 24 hrs icebox
#5	-	-	-	-	-	-	-	-	-	1+	After 30" water bath
	-	-	-	-	-	-	-	-	-	-	After 24 hrs icebox
Guinea Pig											
#1	-	-	-	-	-	-	-	-	-	-	Reading after incubated 30" in water bath at 38 C.and 2 hrs. ice box
	-	-	-	-	-	-	-	-	-	-	
#2	-	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	-	
#3	-	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	-	

Note: In both Tables 1 and 2 the minus sign (-) indicates complete hemolysis and the plus (+) sign indicates amount of "so-called fixation" as in the Wasserman reaction.

TABLE 2

EFFECT OF UREA ON NORMAL DOG ANTI-HUMAN HEMOLYSINS (one unit used)

Serum Number	Tubes numbered from 1 to 7 showing amount (cc) of M/1 Urea used and results.							Remarks
	1	2	3	4	5	6	7	
	0.00	0.02	0.05	0.10	0.20	0.30	0.40	
1	-	-	+	+	3+	4+	4+	
	-	-	-	-	-	-	2+	
2	-	-	-	-	+	2+	4+	The first reading for each serum represents report after 30 min. in water bath at 38 C. The tubes were then placed in ice box for 2 hours and read again as shown in second reading for each serum.
	-	-	-	-	-	-	2+	
3	-	-	-	+	+	3+	4+	
	-	-	-	-	+	1+	3+	
4	-	-	±	±	3+	4+	4+	
	-	-	-	-	-	-	-	
5	-	-	-	3+	3+	4+	4+	
	-	-	-	-	2+	4+	4+	

TABLE 3

SHOWING EFFECT OF INTRAVENOUS INJECTION OF M/1 UREA AND M/1 NaCl ON COMPLEMENT TITRE AND BLOOD UREA OF RABBITS

Material Injected	Time interval in hrs.	Amt. M/1 sol. injected	Blood Urea content Mg.per 100cc	Complement Titre. Amt.of serum containing 1 unit	Remarks
Urea	Before inj.	0.00	55.4	0.07 cc	Determinations made before each injection
	0	2cc			
	1	2cc	60.0	0.08 cc	
	2	2cc	47.3	0.06 cc	
	4	5cc	60.2	0.12 cc	
	5	6cc	69.1	0.085cc	
	6	6cc	60.9	0.10 cc	
	7	0cc	60.0	0.11cc	
NaCl	Before inj.	0 cc	58.2	0.07 cc	
	0	2 cc			
	1	2 cc	54.5	0.07 cc	
	2	2 cc	54.5	0.06 cc	
	4	5 cc	53.7	0.07 cc	
	5	6 cc	54.2	0.06 cc	
	6	6 cc	53.3	0.07 cc	
	7	0 cc		0.07 cc	

An attempt was made to determine the total urea output in urine but I was unable to obtain 24-hour specimens. The quantities obtained from the three urea rabbits varied from 12 c.c. to 21 c.c. The concentrations of urea in terms of 100 c.c. urine were from 1900 to 2400 mg. Underhill and Wells[5] in their work on tartrate nephritis give a normal total volume of urine excreted by a rabbit weighing approximately as much as the three rabbits I used and also given water without food as in my experiments. They injected sodium chloride-urea solution intravenously. From 60 to 80 c.c. were voided normally with a water intake of 40 to 100 c.c.

Table 4

SHOWING THE EFFECT OF INTRAVENOUS INJECTION OF M/1 UREA ON WHITE BLOOD COUNT OF RABBITS

Rabbit Number	Material Injected	Time of Injection in hours	Amt. M/1 Injected	White Cell Count	Remarks
1	Urea	Before Inj	0.00	11,500	This shows result of repeated injections. Determinations made before each injection in all cases.
		0	2 cc.	11,500	
		.5 hr.	2 cc.	9,800	
		1 hr.	2 cc.	7,500	
		2 hr.	2 cc.	3,500	
		3 hr.	0 cc.	6,600	
		4 hr.	2 cc.	9,800	
		4.5 hr.	2 cc.	11,000	
		5 hr.	2 cc.	5,100	
		5.5 hr.	2 cc.	7,500	
2	Urea	Before Inj	0.00	6,100	This shows results of repeated injections.
		0	2 cc.	6,100	
		1 hr.	2 cc.	7,400	
		2 hr.	2 cc.	4,800	
		3 hr.	0 cc.	5,400	
		4 hr.	2 cc.	5,800	
		5 hr.	0 cc.	4,900	
		6 hr.	0 cc.	5,000	
		7 hr.	0 cc.	8,000	
3	Urea	Before Inj	0 cc.	8,900	One injection only
		0 hr.	2 cc.	8,900	
		1 hr.	0 cc.	5,900	
		2 hr.	0 cc.	16,000	
		3 hr.	0 cc.	11,000	
		4 hr.	0 cc.	10,200	
		5 hr.	0 cc.	10,500	
		6 hr.	0 cc.	10,500	
4	Urea	Before Inj	0 cc.	10,400	One injection only
		0 hr.	2 cc.	10,400	
		1 hr.	0 cc.	6,800	
		2 hr.	0 cc.	8,200	
		3 hr.	0 cc.	9,100	

Table 5

SHOWING EFFECT OF INTRAVENOUS INJECTIONS

OF M/1 NACl ON WHITE BLOOD COUNT OF RABBITS

Rabbit Number	Material Injected	Time of Injection	Amt.(cc) of M/1 inj.	White Cell Count	Remarks
5	NaCl	Before	0 cc.	8,900	Effect of Repeated Injections of 2 cc of M/1 NaCl
		0 hr.	2 cc.	8,000	
		1 hr.	2 cc.	6,500	
		2 hr.	2 cc.	9,400	
		3 hr.	0 cc.	9,200	
		4 hr.	2 cc.	8,900	
		5 hr.	0 cc.	8,800	
6	NaCl	Before	0 cc.	10,100	Effect of one injection of 2 cc. of M/1 NaCl
		0 hr.	2 cc.	10,100	
		1 hr.	0 cc.	6,200	
		2 hr.	0 cc.	8,000	
		3.5 hr.	0 cc.	9,000	
		4 hr.	0 cc.	9,500	
		5 hr.	0 cc.	9,800	

Table 6

SHOWING DURATION OF LIFE OF RABBITS

INJECTED WITH HEMOLYTIC STREPTOCOCCI

WITH AND WITHOUT UREA

(0.05 to 0.1 gram urea per Kilo weight of animal added to 24-hour calcium carbonate broth culture of Streptococcus pyogenes just before injection.)

Rabbit Number	Wt. of Rabbit	Number Injections without urea	Number Injections with urea	Duration of life
1	2750		4	18 days
2	2860	4		3 1/3 days
3	1500	2		2 days
4	1550	4		3 1/2 days
5	1400		4	4 days (died with anaphylactoid symptoms as result of intravenous injection)
6	600	3		3 days
7	475		4	30 days
8	580	4		4 days
9	550	4		3 3/4 days
10	575	4		7 days

Note: Parallel series of rabbits receiving streptococcus suspension intravenously in one ear and urea solution in opposite ear all died uniformly within four days.

DISCUSSION: From inspection of the data which is given in Table 1 and 2 it will be observed that complements of different animals differ very markedly in their activity in the presence of urea. Human complement was inactive in the presence of 0.02 to 0.04 c.c. M/1 urea, rabbit and hog complement in the presence of 0.1 c.c., dog complement in the presence of 0.2 c.c., while guinea pig complement did not seem to be materially affected at all. Normal dog hemolysins for human cells as a rule failed to produce hemolysis in the presence of 0.3 to 0.4 c.c. M/1 urea. In a previous communication[1] it was reported that urea did not prevent the union of ambo-ceptor and red blood cells. In the present work I have confirmed my original findings. It seems from a number of experiments which I have done that the so-called "mid-piece" of complement does not combine with the amboceptor-cell complex within the usual incubation period during which time the control tubes were completely hemolysed. In the case of human complement it amounts to the same thing as permanent deviation as will be observed from the above data, but in the case of rabbit, hog, and dog complement it seems that the slowing down of the reactions is the more common observation and that given time enough those complements will ultimately combine and hemolysis result, although frequently it

amounts to permanent deviation. This slowing down effect did not seem to occur with guinea pig complement. It is evident that it does not result because of the amount of serum constituting a unit of complement, as the amount of serum constituting a unit of dog complement, hog complement, or rabbit complement may even equal the amount for a unit of human complement. This slowing down effect seems to vary to some extent with the sample of urea used. In all, four samples of chemically pure urea were used with quantitatively and qualitatively identical results for three samples and a slight quantitative difference for the fourth which gives the slowing down rather than permanent fixing effect observed in the tables for other than human complement but apparently produces permanent deviation in case of human complement. It may be that the slowing down effect is sufficiently slow to permit of the deterioration of complement. One has to consider the possibility of the urea not being absolutely chemically pure. Matthews[6] states that it is by no means impossible that small quantities of cyanamide may be present in urea solutions. In order to determine whether the four samples of urea which I have used contained cyanamide I made use of the qualitative test for cyanamide suggested by Caro, Schuck, and Jacoby and cited by Pranke.[7] This is based upon the observation that the silver salt is an amorphous yellow substance almost insoluble in dilute

ammonia. I found this sufficiently sensitive to detect one part cyanamide in one hundred thousand parts of dilute ammonia water. None of my samples of urea showed any evidence of containing cyanamide. Theoretically the urea used in these experiments was chemically pure.

Blood urea determinations on these respective animals would suggest that there was no correlation between urea content and the inhibitory effect of urea upon complement. Human complement, which was most easily effected, contained less than half of the amount of urea present in rabbit, guinea pig, and hog complement.

This work suggests fundamental differences in the complement of the respective animals although this may be more apparent than real. It would also suggest new evidence of the superiority of guinea pig complement over other complements. The amount required to partially inhibit the action of human complement is very little, if any, greater than the amount of urea present in the blood during severe cases of uremia; however, it is not safe to reason from test tube experiments to the action of urea in the animal body. The results obtained by intravenous injections of M/1 urea are very suggestive, for on the careful perusal of the curves showing complement content compared with blood ureas it will be observed that following injection of

M/1 NaCl there was no accumulation of urea but evidence of diuretic effects was indicated by the initial drop in urea content, that the complement content fluctuated above but never below the initial normal of 0.07 c.c. On the other hand, when M/1 urea was injected there was an initial drop associated with an increase in blood urea followed by a rise associated with relative low blood urea occurring evidently at the time of maximum activity on the part of the kidneys and that two hours later the blood urea had risen from 47.3 to 60.2 mg. per 100 c.c. of blood and corresponding with what appeared to be a very large drop in complement content, and that during the next four hours the complement did not return to normal, and that the low complement content was associated with decreased activity on the part of the kidneys.

This work suggests that a study of the relation of kidney function to complement content might be of value. It is quite evident that in the animals studied equivalent amounts of M/1 NaCl and M/1 urea did not produce identical results. Likewise it will be observed that these two substances did not produce identical results insofar as the blood pictures of the rabbits are concerned. However, in reporting

upon the effect of any substances on the blood counts of rabbits I am aware of a great normal fluctuating variability which tends to lessen the significance of any comparative data. For that reason I would rather merely compare results and suspend judgment. One injection of 2 c.c. of either M/1 urea or M/1 NaCl produces practically identical results, but repeated injections gave different blood pictures. It will be observed that one injection as a rule gave an initial leucopenia followed by a leucocytosis and then returned to normal within an hour or two. Occasionally there will be a slight initial rise followed by a drop with a later rise to normal. Repeated injections, however, produce a leucopenia which persists as a rule during the period of injection and from one to four hours after the last injection. This does not seem to hold as a rule for the injection of similar amounts of NaCl. Unfortunately quantitative blood chloride determinations were not made.

An inspection of the data in Table 4 relative to the effect of urea on streptococcus infection in rabbits brings out some curious and at first apparently contradictory results. It will be noted that those rabbits receiving quantitatively killing doses of a hemolytic streptococcus to which had been added from

0.05 to 0.15 gram of urea, survived by many days those rabbits receiving the same amounts of the same streptococcus without any urea being added to the broth culture. On the other hand, where urea was injected into the opposite ear instead of being added to the broth culture, the animals died as rapidly as did the untreated ones. In view of the fact that the amount of urea used has been shown by others as well as myself not to have any true germicidal effect, I find it difficult to explain these results unless it is that an attenuating or bacteriolytic phenomenon occurs when the urea-streptococcus suspension is suddenly injected into the blood stream in somewhat the same way that urea produced a cytolytic effect as described by Eisenberg[8] and more recently by Mitsuki Kosakai and Coca[9] as follows:

"In order to make a comparative study of the osmotic hemolysis produced by the three selected reagents the concentrations were determined in which they all produce the same degree of hemolysis upon an arbitrarily selected, uniform dimunition of those concentrations. It was found that if 0.05 c.c. of a corpuscular suspension was treated for ten minutes with 0.4 c.c. of 3.5 per cent boric acid or 0.4 c.c. of 4 per cent formaldehyde or 0.8 c.c. of 10 per cent urea,

the total volume in each case being 1 c.c., the sudden addition of 1 c.c. of physiological salt solution would produce very strong hemolysis, while the addition of 2 cc. of that solution would cause complete hemolysis in all of the mixtures."

They explain this curiously indirect effect of urea on the basis of osmotic changes. I have confirmed their work as to the action of these various reagents upon sheep red blood cells and have also noted that the same phenomenon occurs when beef cells were used, but that human cells and rabbit cells do not lake under the above stated conditions. If the concentrations of urea and the other reagents are increased sufficiently laking of human cells and rabbit cells can be obtained by the same technique. It is conceivable that such a phenomenon is responsible for the results which I obtained. It would seem that there would be as great a variation among bacteria as among red blood cells in their resistance to cytolysis. Furthermore it is conceivable that an attentuating effect may occur without actual cytolysis. This is under investigation at the present time and will be reported later.

It is rather interesting to note from the temperature curves of the so-called treated animals

that when they were taken the temperature which they developed as the result of the inoculation was markedly modified by the intravenous injection of 0.05 to 0.15 gram of urea. This may be entirely due to the diuretic action of urea although it may be due to some stimulation of the immunity mechanism of the body.

Urea seems to be quite widely distributed in nature. Fosse[10] reports its presence in all living matter but does not offer any suggestion as to its uses. Very little seems to have been done to determine the exact physiological and pharmacological action of urea. Mathews[11] summarizes the current conception as to origin and physiological action as follows:

"In the first instance it is undoubtedly formed from ammonia... But ammonia is not the only substance which can serve as the precursor of urea and the same is true of any amino acid and many other substances which contain amino nitrogen... In some animals too although it is doubtful whether it occurs in man, there is an oxidation and hydrolysis of uric acid to urea. This is probably, if it occurs, an unimportant source of human urea."

As to the physiological action of urea he states further as follows:

"Urea has several quite important physiological actions. In the first place it is the natural diuretic... It is possible that by stimulating the secretion of urine the drinking of more water results and a kind of catharsis of the cells of the body might be produced which might be either advantageous or disadvantageous to them. Urea has also a very definite function in the cells of the elasmobranch fishes and possibly in the mammalia also. It is one of the normal constituents of the cell and of the blood and other body fluids and since these cells have for long years of time been selected to work with the highest degree of efficiency in this urea containing medium it is found that the addition of a little urea to artificial perfusion solutions when one is perfusing the heart or other organs, is as a rule advantageous. The effect of such an addition to the salt solutions used to sustain the heart beat of fishes both teleosts and elasmobranchs is very marked. Tissues live much longer in the presence of some urea than in its absence. The effect on mammalia is much less marked since the amount of urea in the blood of mammals is very small, 0.02 to 0.04 per cent. The effect produced by the addition of urea to the artificial salt solutions in the elasmobranchs is that of stimulation

and the same effect may be produced by the addition of smallamounts of ammonium carbonate. It is possible that in these animals there may be some conversion of urea into ammonium carbonate or vice versa and the effect may be due to the action of ammonium carbonate in neutralizing acids. Urea is not then entirely inert."

Von Furth[12] summarizes the work of Heilner[13] on the physiological reaction of urea as follows:

"Urea introduced subcutaneously has a stimulative effect on protein metabolism thus suggesting the possibility of urea itself being a factor in the special mechanism regulative of the course of intracorporeal protein disintegration."

Eyster states that urea has a stimulating action on the heart.[14] It would appear from Solis-Cohen, Kolmer and Heist's work that urea materially contributed to the efficiency of quinine-urea in the treatment of pneumococcus infections. It may be that the results of Symmers and Kirk, who recommend the use of urea in the treatment of war wounds, are due entirely to the germicidal properties of urea in very strong concentrations, although their work suggests that it would be worth while to study the effects of urea on phagocytosis. In a similar manner we are studying the effects of uric acid and purine ring compounds

upon immunity reactions. For various reasons urea seems to warrant consideration at the present time.

Summary and conclusions of the present paper may be stated as follows:

(1) Urea solutions slow down the union of complement with amboceptor-cell complex.

(2) The slowing down effect varies for different complements ranging from what amounts to permanent deviation for human complement to no appreciable effect for guinea pig complement.

(3) In test tube experiments it requires ten times as much M/1 urea to inhibit rabbit and seven times as much for hog complement and ten times as much for dog complement as it takes for human complement.

(4) Urea in NaCl solution in the concentrations stated does not directly lake red blood cells or interfere with the union of amboceptor and red blood cells.

(5) The laking phenomenon observed by Mitsuki Kosakai and Coca for sheep cells was not observed using the same concentrations of urea for human cells or rabbit cells, but was found to hold true for beef cells. This phenomenon may be obtained for human cells or rabbit cells if the concentrations of urea are sufficiently increased in the preliminary treating sol-

ution.

(6) The intravenous inoculation of from 2 to 6 c.c. M/1 NaCl solution in rabbits gave a slight diuretic effect but no noticeable effect upon complement.

(7) Repeated injections of similar amounts of M/1 NaCl produced a slight initial leuko - penia followed by leukocytosis in rabbits.

(8) One injection gave one initial leukopenia lasting for two hours followed by a return to normal.

(9) Repeated injections of M/1 urea solution was associated with a decrease in complement content.

(10) The slight rise in complement two hours after the initial injection was associated with the maximum activity on the part of the kidneys.

(11) Repeated injection of M/1 urea was associated with wide fluctuations in the blood count but on the whole produced noticeable leukopenia.

(12) One injection of 2 c.c. M/1 urea gave an initial leukopenia followed by a marked leuko- cytosis, the blood count returning to normal inside of three hours and remaining normal.

(13) The duration of life of rabbits injected with killing doses of hemolytic streptococci

to which had been added from 0.05 to 0.15 gram of urea was markedly prolonged and that this was perhaps partially if not wholly due to an attenuating or cytolytic phenomenon similar to the one observed by Kosakai and Coca.

(14) Similar amounts of urea when injected in the opposite ear vein apparently did not protect the animals.

(15) In streptococcus infections the intravenous injection of urea seemed to have an effect upon temperature of the animals.

Plate I

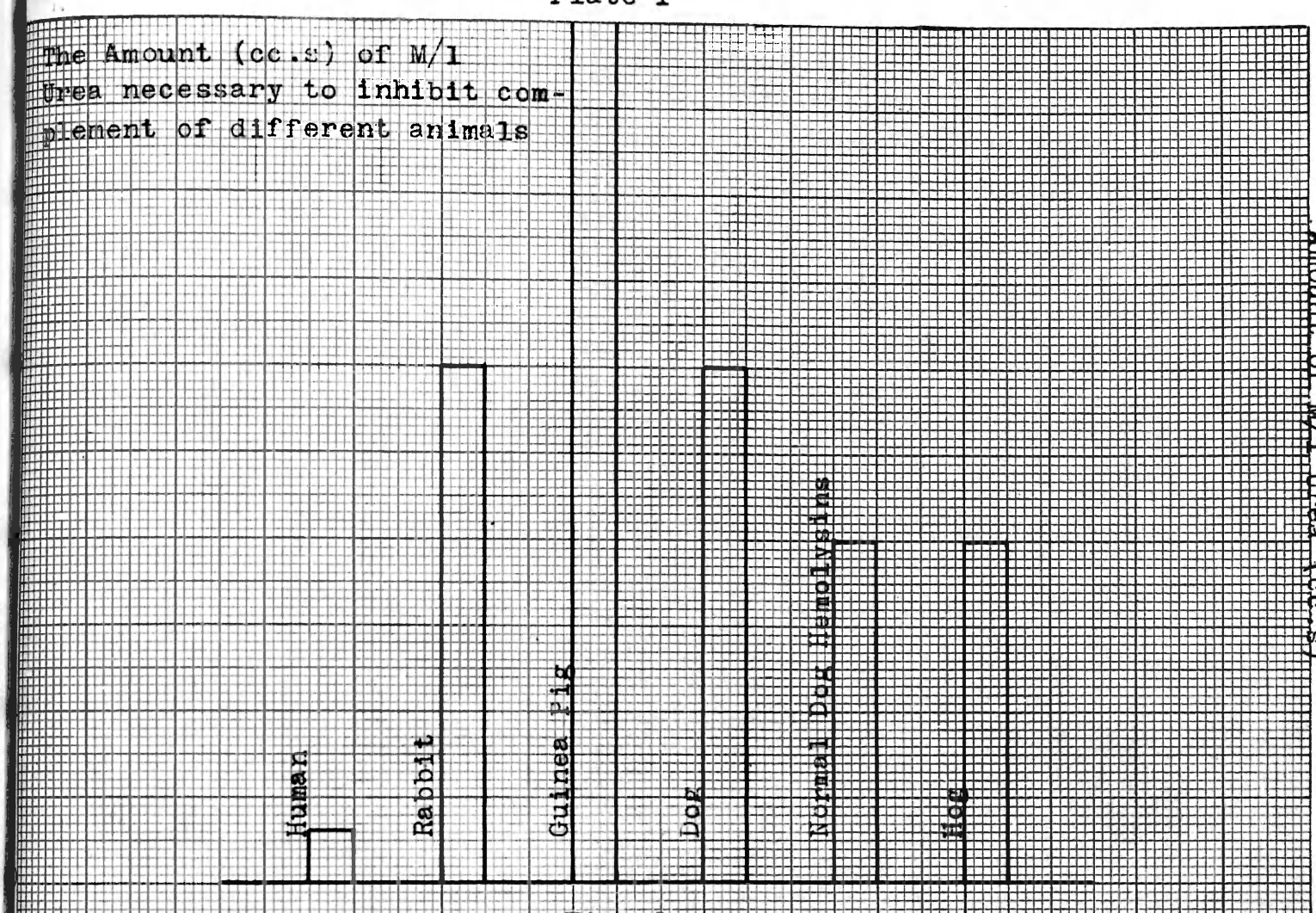


Fig. 1

The Effect of I.V.Injection of M/1 NaCl and M/1 Urea on Complement Titre

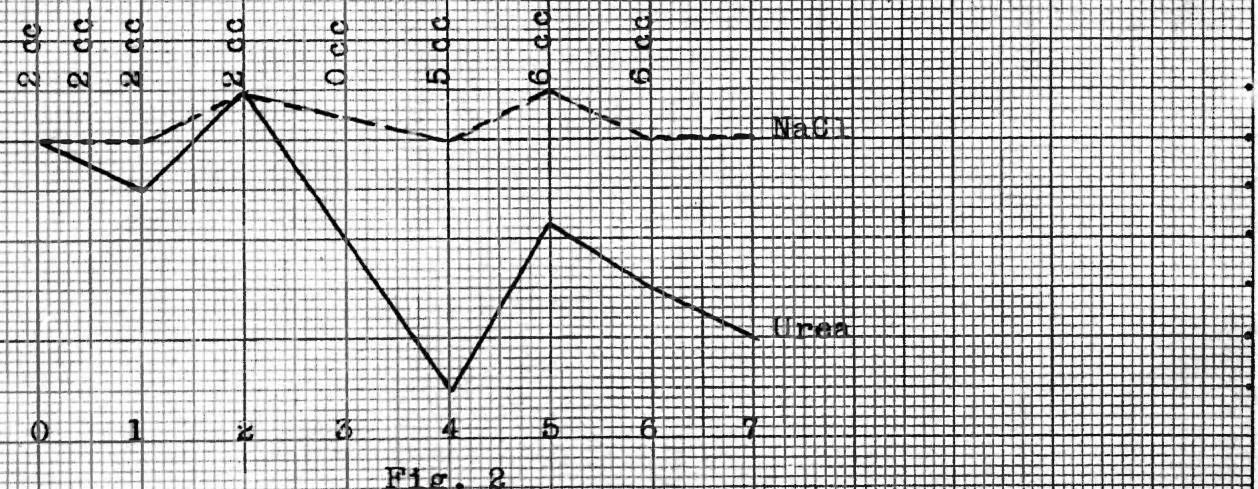


Fig. 2

Legend - Broken line gives titre following NaCl injection
Solid line shows titre following Urea injection

Plate 2

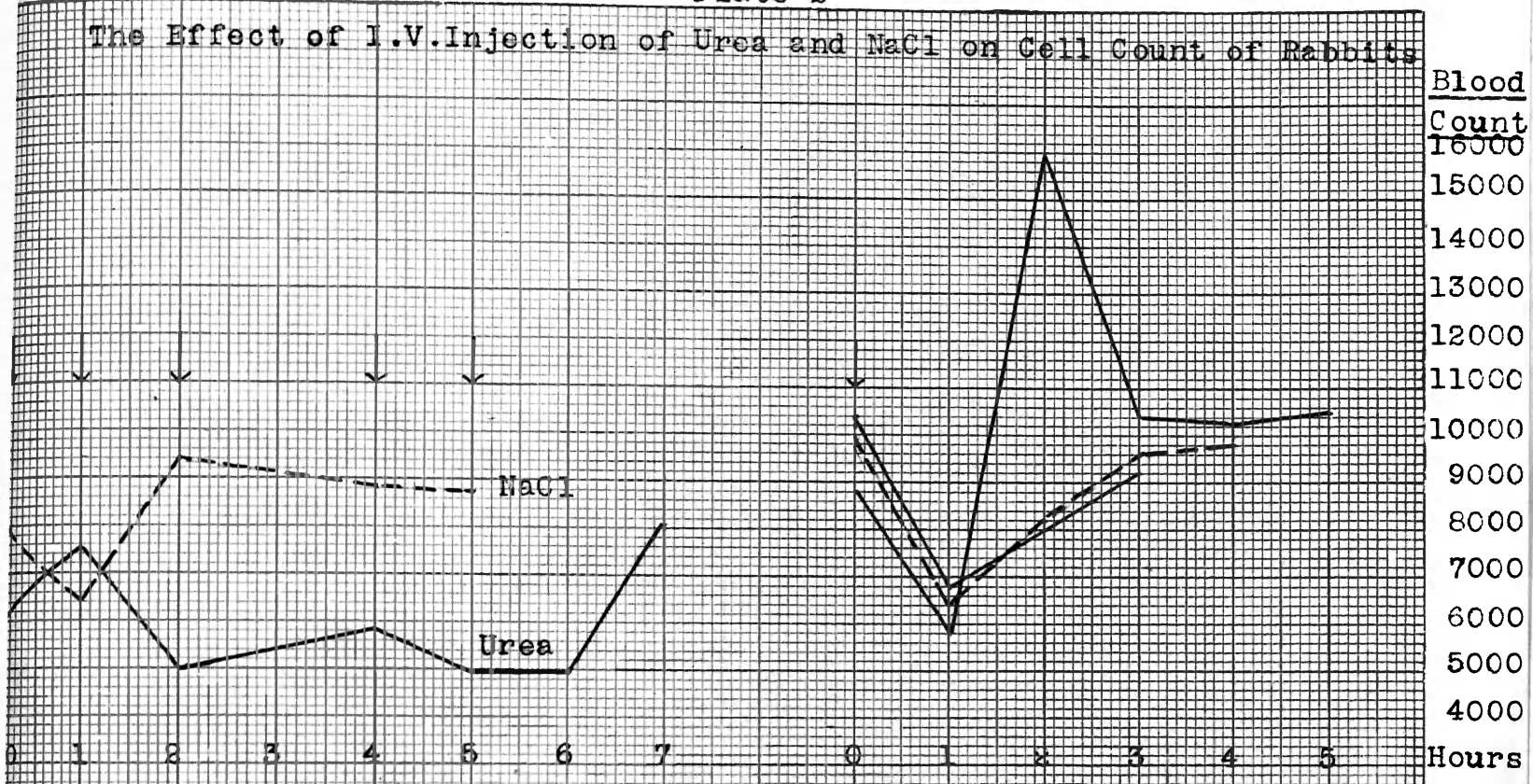


Fig. 1 (Repeated Injections)

Fig. 2 (One Injection)

Legend-Injection of 2 cc. of M/1 solution indicated by arrow.
Solid line shows curve for Urea rabbit.
Broken line shows curve for NaCl rabbit.

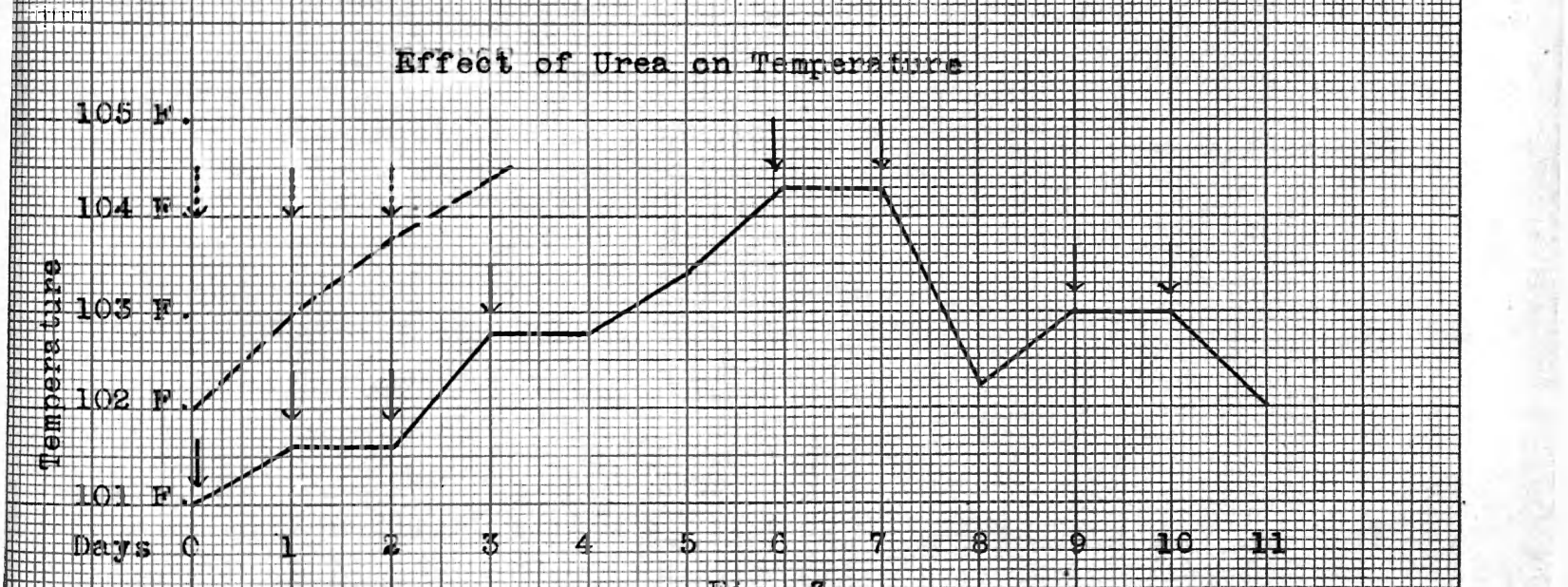


Fig. 3

Legend - Broken line shows temperature of rabbit receiving streptococcus suspension without Urea
Solid line for rabbit receiving 4 injections of streptococcus-urea suspension and afterward urea only as indicated by arrow ( )

BIBLIOGRAPHY

1. Sherwood, Jour. Infect. Dis., 1917, Vol. 20, #2, p. 185-200.
2. Symmers and Kirk, Lancet, 1915, Vol. 11, p. 1237.
3. Solis-Cohen, Kolmer and Heist, Jour. Infect. Dis., March 1917, Vol. 20, #3, p. 245-333.
4. Gradwohl, The Newer Methods of Blood and Urine Chemistry, 1917, C.V.Mosby & Co. Pub., p. 42-46.
5. Underhill and Wells, Jour. Exp. Med., Vol. 18, #4, 1913, p. 347-352.
6. Matthews, Physiological Chemistry, 2nd edition, p. 699.
7. Pranke, Cyanamid, 1913, Chemical Pub. Co., p. 17.
8. Eisenberg, Centralb. f. Bakt., Orig. 1913, 69, p. 173.
9. Kosakai, Jour. Immunology, March 1919, Vol. 4, #2, p. 52.
10. Fosse, Annals de L'Institute Pasteur, Oct. 1916, Vol. XXX, #10, p. 525-592.
11. Matthews, Physiological Chemistry, 2nd edition, p. 699, 701.
12. Von Furth, Chemistry of Metabolism, Trans. by Allen J. Smith, 1916, p. 499.
13. Heilner, Zeitschr, f. Biol. 52, 216, 1909.
14. Eyster, Science, 1910, Vol. 31, p. 236.

In summing up the work of this thesis it is apparent that the interference with liver function by the anastomosing of portal circulation with the vena cava was not associated with the progressive drop in complement although there was progressive atrophy of the liver associated with fatty degeneration. It will be apparent that a drop in complement occurred during the operation and that the complement content was low for several hours afterward. The recovery of the animals was associated with a return to normal complement content. This drop in complement is similar to the results obtained by Nolf except that his animals died four hours after the operation. It also corresponds to the drop obtained by Ehrlich and By Dick, produced by necrosis of the liver by various means. Animals so treated however did not survive many hours. It is quite reasonable to believe that the phosphorus poisoning of Ehrlich and chloroform and hydrazin sulfate poisoning of Dick had a profound effect upon tissue cells and their metabolism throughout the rest of the body as well as the very pronounced effect upon liver tissue.

The results which are reported in this thesis would seem then to give results less open to criticism than the results of the other work. The hepatic circulation was not impaired in these experiments because

it was felt that the animals would not long survive if the livers were practically extirpated. It was felt that the very decided interference with liver function should be associated with at least a noticeable lowering of complement content in case the liver was the sole source of complement production and that the study of complement content compared to changes in the liverwould have some significance. In attempting to throw some light upon the temporary drop which did occur the author was lead to conduct the experiments as outlined in Studies II. He feels that the results would suggest that the temporary drop was due to a number of factors rather than any individual thing.

Many of these factors might be enumbrated--the effect of the prolonged state of surgical anesthesia and the blood and tissue chemical changes associated therewith, the presence of the anesthesia in the circulating fluids and the rather unknown condition called shock, also the impairment of such a large functioning gland as the liver which may produce its quota of complement. It would seem that other tissues of the body have the ability to maintain complement content to the normal level so long as the animal organism is in a comparatively satisfactory state of health. It has also been brought outthat one must be careful in drawing conclusions from the experi-

mental results upon one type of animal and forming general conclusions therefrom, because it has been shown that urea, for example, is quantitatively different in its action on complement for the various animals, whereas hydrochloric acid is uniformly very much of an inhibiting or destructive agent to all of the complements studied.

Another interesting and suggestive observation which has come from this work is that a majority of the substances studied when used in concentrations too small to interfere with complement in the test tube experiments would give a marked accelerating and augmenting effect upon the hemolytic reaction. This may throw some light upon the so-called "third compnnent" of a complement which has been studied quite extensively by Manwaring. Quantitative studies of results obtained in the course of these experiments have been confirmed by Kolmer who has made use of this material in studying the possible errors in complement fixation work.

It is also possible that this work may throw some light upon some of the cases of local lowered resistance of tissue to infection. There are in addition to these salient points of the thesis many other observations of interest which may be found in greater detail in the context.

ACKNOWLEDGMENTS

This work was started and completed under the direction of Prof. Frederick H. Billings, Head of the Department of Bacteriology in the University of Kansas until 1918. To him the author is greatly indebted for the opportunity of carrying out the research work and for his many helpful suggestions and encouragements. The author wishes further to express his appreciation for the many helpful criticisms of this work offered by Dr. S. A. Matthews, Professor of Physiology of the University of Kansas. He is indebted to Dr. F. B. Dains, Professor of Organic Chemistry at the University of Kansas for many of the chemicals used, and to F. H. Brueckmiller of the Department of Chemistry, University of Kansas, for helpful suggestions in regard to the hydrogen-ion concentration work the author is also indebted.